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EXAMINER	
FALK, ANNE MARIE	

ART UNIT	PAPER NUMBER
1632	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/040,518	KARATZAS ET AL.	
	Examiner	Art Unit	
	Anne-Marie Falk, Ph.D.	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *11 February 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *22-36 and 39-58* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *22-36 and 39-58* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

The amendment filed February 11, 2004 (hereinafter referred to as "the response") has been entered. Claims 39-41 and 54-57 have been amended. Claims 37 and 38 have been cancelled.

Accordingly, Claims 22-36 and 39-58 remain pending in the instant application.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 22-36 and 39-58 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Applicants are referred to the final guidelines on written description published January 5, 2001 in the Federal Register at Volume 66, Number 4, pp. 1099-1111 (also available at www.uspto.gov).

Vas-Cath Inc. V. Mahurkar, 19 USPQ2d 1111, clearly states that "applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. The invention is, for purposes of 'written description' inquiry, whatever is claimed" (see page 1117). Applicant is reminded that *Vas-Cath* makes clear that the written description provision of 35 U.S.C. 112 is severable from its enablement provision.

Claims 22-36 and 54-58 are directed to nucleic acid molecules comprising a nucleotide sequence encoding a biofilament polypeptide and a regulatory sequence that directs expression of a polypeptide in milk-producing cells of a ruminant, wherein said regulatory sequence is operably linked to said nucleotide

sequence, and wherein said biofilament polypeptide comprises a leader sequence that enables secretion of said biofilament polypeptide by said milk-producing cells into milk of the ruminant. The claims encompass a large genus of nucleic acid molecules encoding a large genus of biofilament polypeptides, but the specification does not describe a representative number of nucleic acids encoding biofilament polypeptides. The specification defines "biofilament" as "a fibrous protein that is normally produced and secreted by any one of a variety of insects and arachnids" (page 5, lines 6-7). The prior art describes the DNA sequence of *Bombyx mori* fibroin gene (Tsujimoto et al., 1979). While the claims cover a very large genus of nucleic acid molecules, the specification only describes a few nucleic acids that encode biofilament polypeptides. There are over 34,000 known species of spider, each making its own silk. Each spider species can make up to seven different types of silk fibers. The specification describes partial cDNAs encoding 2 biofilaments from *Nephila clavipes* (spidroin-1 and spidroin-2) and 4 biofilaments from *Araneus diadematus* (ADF-1, ADF-2, ADF-3, and ADF-4) (see specification at pages 9-10 and Table 1). The partial cDNAs encode proteins that are shorter than the native proteins. However, the claims cover a large genus of nucleic acid molecules from a wide variety of insects and arachnids (as well as the full-length forms of the native proteins), the vast majority of which are not described by the instant specification. Thus, the specification fails to describe the entire genus of nucleic acid molecules encoding a biofilament polypeptide, as recited in the claims. While the specification describes a 47 amino acid consensus sequence from *Nephila clavipes* spidroin-2, designated SEQ ID NO: 3, and a 34 amino acid repeat motif of *Nephila clavipes* spidroin-1, designated SEQ ID NO: 2, the specification does not teach what distinguishing features are shared by other members of this genus. In analyzing whether the written description requirement is met for genus claims, it is first determined whether a representative number of species have been described by their complete structure. In the instant case, only 6 biofilament polypeptides from 2 species of spiders out of 34,000 species have been described in sufficient detail to permit construction of a nucleic acid construct as claimed. Next then, it is determined whether a

representative number of species have been suffficiently described by other relevant identifying characteristics. In this case, although the claims cover any nucleic acid encoding a biofilament polypeptide secreted from an insect or arachnid, coding nucleic acids other than the 6 described in the specification have not been described by relevant identifying characteristics. This limited information is not deemed sufficient to reasonably convey to one skilled in the art that Applicants were in possession of the entire genus of nucleic acids encoding biofilament polypeptides covered by the claims, at the time the application was filed. Thus, it is concluded that the written description requirement is not satisfied for the claimed nucleic acids, animals comprising the nucleic acids, and mammary epithelial cells comprising the nucleic acids.

Claim 39 is directed to a transgenic female ruminant comprising mammary tissue cells that comprise the nucleic acid molecule of claim 22 (a nucleic acid encoding a biofilament polypeptide), wherein the ruminant secretes a biofilament polypeptide into milk. Claims 40 and 42-53 are directed to methods of using the female ruminant of Claim 39 to produce a biofilament polypeptide. The claims continue to cover a female ruminant produced by *in vivo* somatic cell gene transfer. However, *in vivo* somatic cell gene transfer is not described or contemplated in the specification. The specification only describes a transgenic ruminant comprising the transgene in all its somatice and germ cells. As understood in the art, a transgenic animal comprises a transgene within its genome and the transgene is present in all somatic and germ cells of the animal. The specification fails to describe the entire genus of female ruminants comprising mammary tissue cells that comprise the nucleic acid molecule of Claim 22 as claimed. The specification does not teach what distinguishing features are shared by members of this genus. In analyzing whether the written description requirement is met for genus claims, it is first determined whether a representative number of species have been described by their complete structure. In the instant case, only transgenic ruminants comprising a transgene within the genome of all somatic and germ cells are described by their complete structure. Next then, it is determined whether a

representative number of species have been suffficiently described by other relevant identifying characteristics. In this case, although the claims cover animals produced by *in vivo* somatic cell gene transfer, no such species have been described by relevant identifying characteristics. This limited information is not deemed sufficient to reasonably convey to one skilled in the art that Applicants were in possession of the entire genus of female ruminants covered by the claims, at the time the application was filed. Thus, it is concluded that the written description requirement is not satisfied for the claimed animals and methods of using said animals.

At page 8 of the response, Applicants argue that a founder transgenic animal may be chimeric and that in such a case the transgene would not be present in all somatic and germ cells. It appears that Applicants are speaking of **mosaic** animals, not **chimeric** animals. The only technique discussed in the application for producing transgenic animals is pronuclear microinjection. Pronuclear microinjection does not lead to the formation of chimeric animals. Chimeric animals are animals that possess cells from at least two different strains or individuals. **Chimeric** animals are formed when cells from one strain are injected into the blastocyst of another strain. **Mosaic** animals are formed when pronuclear microinjection is used to produce transgenic animals and several cell divisions occur before integration of the transgene into the genome. In such a case, not all cells of the animal will comprise the transgene. There may or may not be germ cells that comprise the transgene. The instant specification does not describe nor does it teach how to use mosaic animals for the asserted utility of producing biofilament polypeptides in the milk in quantities sufficient for isolation of the biofilament protein.

Claims 22-36, 39, 40, and 42-53, and 54-58 rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Upon further consideration, the enablement rejection is reinstated as applied to the claims directed to the transgenic female ruminant, the methods of using the transgenic female ruminant, and nucleic acid claims that recite an intended use.

Claims 22-36 and 54-58 are directed to nucleic acid molecules.

Claim 39 is directed to a transgenic female ruminant.

Claims 40 and 42-53 are directed to a method of using the transgenic female ruminant for producing a biofilament polypeptide. The claims include isolating the biofilament polypeptide from milk.

The Declaration of Dr. Karatzas, filed April 18, 2002, states that the results described therein were obtained "using the methods described in the patent application." Numerous experiments involved the use of **nuclear transfer** for the production of the transgenic goats described therein. However, the specification does not disclose using nuclear transfer for generating transgenic ruminants, but rather only discloses using pronuclear microinjection. Paragraph 4a of the Declaration states that three founder goats were produced by pronuclear microinjection (using a WAP promoter/spider silk ADF-3 transgene). The Declaration further states that, within the F1 generation, the ADF-3 biofilament protein was confirmed by tests that included Western blot analysis to be present in the milk. However, the Declaration does not report the quantity of biofilament protein present in the milk. This is at issue because the asserted utility of the animal is for the isolation of the biofilament protein from the milk. Thus, the specification must provide an enabling disclosure for producing sufficient quantities of biofilament protein to permit isolation from the milk. Therefore, the enablement rejection is reinstated as set forth below.

The specification fails to provide an enabling disclosure for the preparation of a transgenic ruminant of the type claimed because the phenotype of a transgenic animal cannot be predicted. Furthermore, the specification fails to provide an enabling disclosure for the preparation of the broad scope of transgenic ruminant species of the type claimed. The specification does not teach how to obtain sufficient amounts of biofilaments from the claimed ruminants. The specification only teaches the

anticipated expression of the transgene, but the specification does not offer adequate guidance to teach one skilled in the art how to produce a transgenic cow, goat, or sheep that expresses a biofilament in milk to a level sufficient to allow purification of the biofilament from the biological fluid. The mere capability to perform gene transfer in any given species is not enabling for the claimed transgenic ruminants because the desired phenotype (in this case, the expression of a biofilament in milk at a level sufficient to permit isolation and purification of the biofilament) cannot be predictably achieved simply by introducing transgene constructs of the type recited in the claims. While gene transfer techniques are well-developed for a number of species, especially the mouse, methods for achieving the desired level of transgene expression in appropriate tissues are less well-established. The introduction of DNA into the mammalian genome can ordinarily be achieved most reliably by microinjection or retrovirus-mediated gene transfer. However, the state of the art for transgenics is unpredictable because the method of gene transfer typically relies on random integration of the transgene construct. Insertional inactivation of endogenous genes and position effects (see Wall, 1996, p. 61, paragraph 3) can dramatically influence the phenotype of the resultant transgenic animal. Integration of the transgene near highly active genes or, alternatively, in a transcriptionally inactive region, can influence its level of expression. Furthermore, expression of the transgene and the effect of transgene expression on the phenotype of the transgenic animal depends on the particular gene construct used, to an unpredictable extent. The particular genetic elements required for appropriate expression varies from species to species. Thus, a construct that confers the desired phenotype in a mouse will not necessarily achieve the same result in a rat. Wall (1996) reports that our lack of understanding of essential genetic control elements makes it difficult to design transgenes with predictable behavior (p. 61, paragraph 3). This is especially relevant for species in which genetic studies are less advanced than in the mouse. Thus, the species-specific requirements for transgene design introduces an additional level of unpredictability associated with the development of transgenic animals. Furthermore, there are inherent physiological differences between mice, goats, cows, sheep, etc. that can

affect the phenotype in an unpredictable manner. In the absence of specific guidance, the existence of any phenotypic alteration resulting from the introduction of a nucleic acid construct comprising a biofilament gene operably linked to a milk-specific promoter in any ruminant species is highly unpredictable. Given the lack of working examples and the unpredictability in the art, one of ordinary skill in the art would have been required to engage in undue experimentation in order to make and use the claimed transgenic ruminants.

The specification fails to provide an enabling disclosure for the claimed nucleic acid constructs because the constructs are not enabled for the intended use for the reasons discussed herein above. The claims recite an intended use, wherein the leader sequence "enables secretion of said biofilament by said milk-producing cells into milk of the ruminant." However, for the reasons discussed above the specification is not enabling for the use of the nucleic acid construct to produce transgenic ruminants that express a biofilament in the milk.

Given that specific phenotypic alterations cannot be predictably achieved by merely transferring a gene of interest into an animal, specific guidance must be provided in the disclosure to enable the instant invention. The specification must teach those skilled in the art how to make and use the full scope of the claimed invention without undue experimentation. The claims cover any transgenic ruminant harboring any construct of the type recited in the claims, but the specification does not enable such animals nor the use of such animals in the claimed methods. In the absence of disclosure of a transgenic animal exhibiting the appropriate phenotype, undue experimentation would have been required to make and use the claimed animals.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 22-36, 41-53, and 54-58 are rejected under 35 U.S.C. 103(a) as being unpatentable over Huynh et al. (1991, Experimental Cell Research 197: 191-199), U.S. Patent No. 5,227,301 (Turner et al., 1993), Fahnestock et al. (1997, Appl. Microbiol. Biotechnol. 47: 23-32), and Ebert et al. (1994, Bio/Technology 12: 699-702).

Claims 41-53 are directed to a method for producing a biofilament polypeptide by culturing a mammary epithelial cell comprising a nucleic acid construct, under conditions in which said biofilament polypeptide is expressed and secreted into a culture medium. The nucleic acid construct comprises a nucleic acid encoding a biofilament polypeptide operably linked to a regulatory sequence that directs expression of a polypeptide in milk-producing cells of a ruminant, wherein said biofilament polypeptide comprises a leader sequence that enables secretion of said biofilament polypeptide by said milk producing cells into milk of the ruminant.

Claims 22-36 and 54-58 are directed to nucleic acid molecules.

Huynh et al. (1991) and Turner et al. (1993) disclose a clonal cell line produced from primary bovine mammary alveolar cells (MAC-T) by stable transfection with a vector encoding SV40 large T antigen. The immortalized cells do not exhibit a transformed phenotype. The cells are responsive to lactogenic hormones (p. 198, column 2 of Huynh et al.). When differentiated, the cells synthesize and secrete α- and β-casein. Thus, the phenotype of the cells makes them suitable as an *in vitro* model for bovine lactation. Turner et al. explicitly points out that the cell line can be used in a method for indefinitely expressing foreign genes (see abstract and Example III at Column 10). Turner et al. also

points out that eukaryotic fermentation is a viable means of overcoming the considerable problems associated with prokaryotic expression (column 10), since eukaryotic proteins require posttranslational modification and the proper folding environment (column 10).

Fahnestock et al. (1997) disclose the production of synthetic spider dragline silk proteins in *Escherichia coli*. The expression system is not ideal for expressing eukaryotic proteins, as acknowledged at page 30, column 2, paragraph 5. The experiments performed revealed a number of difficulties associated with expression of the synthetic spidroin genes, including truncated synthesis, poor codon adaptation, and genetic instability. The authors further noted that the spidroin-1 and spidroin-2 genes are highly repetitive at the DNA level and are both poorly adapted to expression in *E. coli*.

Ebert et al. (1994) disclose the production of a human recombinant protein in the milk of transgenic goats. The human cDNA was inserted between exons 2 and 7 of the goat β-casein gene. Lactation was induced and milk containing the human recombinant protein was harvested.

Since spider silk proteins have desirable properties, such as high tensile strength and elasticity, skilled artisans are clearly interested in producing these proteins in large quantities, as evidenced by the work of Fahnestock et al. Since the cell line disclosed by Huynh et al. has been shown to synthesize and secrete milk proteins into culture media, one of skill in the art would recognize that the cells disclosed by Huynh et al. (1991) are ideal for expressing recombinant proteins that will be secreted directly into the culture media, which would clearly facilitate isolation of the recombinant protein. Moreover, given the problems revealed in attempting to express synthetic spider dragline silk proteins in *E. coli*, one of skill in the art would have realized that a eukaryotic expression system would be more compatible for expression of eukaryotic genes. In view of the successful expression of human tissue plasminogen activator in the mammary gland of transgenic goats, the skilled artisan would have readily recognized the advantages of expressing silk proteins in mammary epithelial cells in culture. Thus, it would have been obvious to one of skill in the art to have used a β-casein construct analogous to that depicted in Figure 1 of Ebert et al.

for insertion of the *Nephila clavipes* synthetic genes described by Fahnestock et al. The resulting construct would be used to transfect the mammary epithelial cell line of Huynh et al. One of skill in the art would have anticipated a reasonable expectation of success because the desired cell line was readily available and had already been shown to have the necessary phenotype, *i.e.* it is responsive to lactogenic hormones and secretes milk proteins.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Anne-Marie Falk whose telephone number is (571) 272-0728. The examiner can normally be reached Monday through Friday from 10:30 AM to 7:00 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Amy Nelson, can be reached on (571) 272-0804. The central official fax phone number for the organization where this application or proceeding is assigned is (703) 872-9306.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to Dianiece Jacobs, whose telephone number is (571) 272-0532.

Anne-Marie Falk, Ph.D.

Anne-Marie Falk

ANNE-MARIE FALK, PH.D
PRIMARY EXAMINER